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14. ABSTRACT
The burden of traumatic brain injury (TBI) is expressed in the disabling behavioral and cognitive abnormalities noted in significant number of combat veterans. These clinical phenotypes suggest impairment in distributed cerebral functions dependent on the integrity of white matter (WM) tracts. In this proposal we explore mechanisms of mild blast trauma-associated demyelination, with the goal of testing a therapeutic strategy to enhance remyelination using human glial restricted progenitors (hGRPs; Q Therapeutics Inc.).

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1. Introduction

The burden of traumatic brain injury (TBI) is expressed in the disabling behavioral and cognitive abnormalities noted in significant number of combat veterans. These clinical phenotypes suggest impairment in distributed cerebral functions dependent on the integrity of white matter (WM) tracts. In this proposal we explore mechanisms of mild blast trauma-associated demyelination, with the goal of testing a therapeutic strategy to enhance remyelination using human glial restricted progenitors (hGRPs; Q Therapeutics Inc.).

2. Keywords

Myelin; Blast Injury; MRI; Stem Cells

3. Accomplishments (supervising PI listed in the brackets):

What were the major goals of the project?

The overall goal of this project is to assess utility of glial progenitors for treatment of traumatic (blast) brain injury. Specific goal for the first phase of the study is to characterize mouse model of blast injury putting emphasis on the status of white matter.

What was accomplished under these goals?

During the first year of grant duration we accomplished the following:

1. Obtaining IACUC and ACURO approvals at The Johns Hopkins University and Walter Reed Army Institute of Research (Dr. Janowski)
2. Selection, hiring and providing appropriate training for post-doctoral fellow (Drs. Walczak and Janowski)
3. Short-term study with optimization of blast dose in mice
    a. Evaluation of blast overpressure dosage for the mice strain (BALB/c) with regard to mortality (Dr. Janowski)
    b. Assessment of acute behavioral outcomes following various doses of blast overpressure (Dr. Janowski)
    c. Assessment of acute MRI outcomes following various doses of blast overpressure with regard to focal changes (Dr. Walczak)
    d. DTI MRI studies were performed for animals injured with optimized dose of 20 psi and data were analyzed (Dr. Walczak)
    e. Animal plasma has been secured and it is currently under analysis (Dr. Janowski)
    f. Gross morphological changes from different dosages of blast over pressure were studied using ventricular volume analysis on MRI
    g. Immunohistochemistry on mouse brains was performed to detect acute cell death in different dosages of blast overpressure (Dr. Janowski)
4. Long-term blast study
    a. We encountered an issue with reproducibility of blast overpressure dosage. In first round of experiments we identified that 20psi is a dose resulting with low and acceptable mortality; however, in second cohort we observed unacceptably high mortality rate. This may be related to the change of personnel at WRAIR performing the blast procedure. Following further optimization of the blast experimental set-up we found a new optimal dosage at 17*2 psi for our studies and that proved to be reproducible and with minimal mortality rate (Dr. Janowski)
    b. Blast dose of 17*2 has been successfully applied for long-term study (Dr. Janowski)
    c. Long-term studies are currently underway with the acquisition of longitudinal DTI to understand dynamics of demyelination following blast overpressure exposure (Dr. Walczak)
    d. Temporal assessment of learning and memory behavior battery studies are currently underway (Dr. Janowski)
    e. Analysis and cross-correlation of acquired DTI and behavioral data is currently underway (Dr. Walczak)

Reportable Outcomes:

1. Short-term blast dose finding study in mouse

1. Mortality assessment (Dr. Janowski):

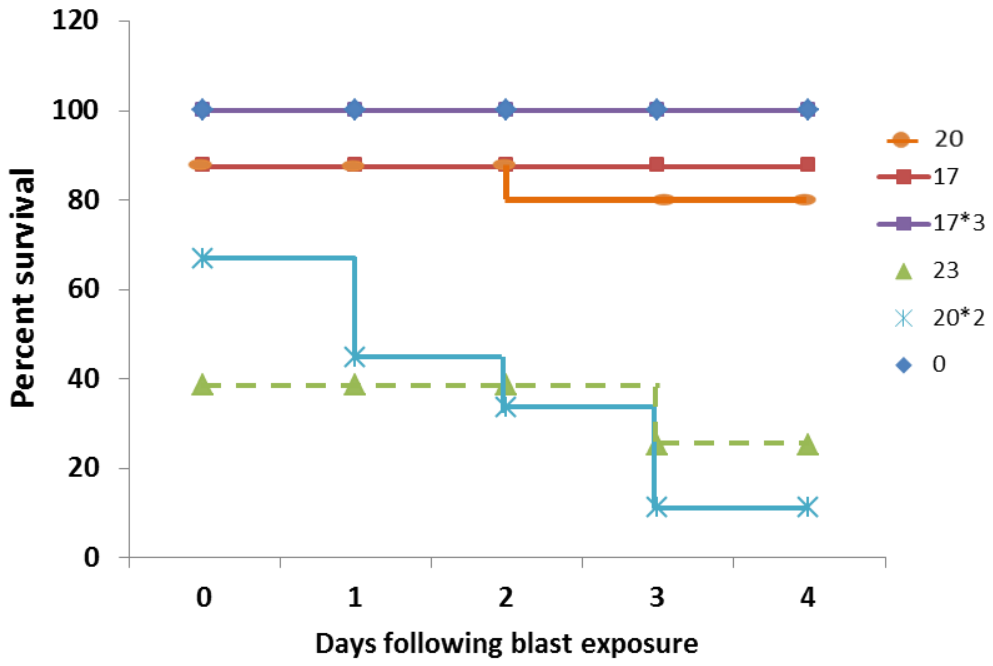

Figure 1: Time course assessment of survival rate following blast overpressure at various different intensities

2. Righting reflex *vs* mortality (Dr. Janowski)

The time of righting reflex following the injury provides an overall estimate of the injury following blast overpressure. Previously published reports (Long et al., 2009) have shown blast overpressure (BOP) exposed groups had a significant increase in righting time using a rat model following BOP. In order to access the relative outcomes compared to rat model, we have assessed the righting reflex time following BOP in mice (strain: BalB/c) model. In accordance with previous reports of rat animal model, significant increase in righting reflex time ($p < 0.05$) was found in all groups following BOP exposure (Figure 2).

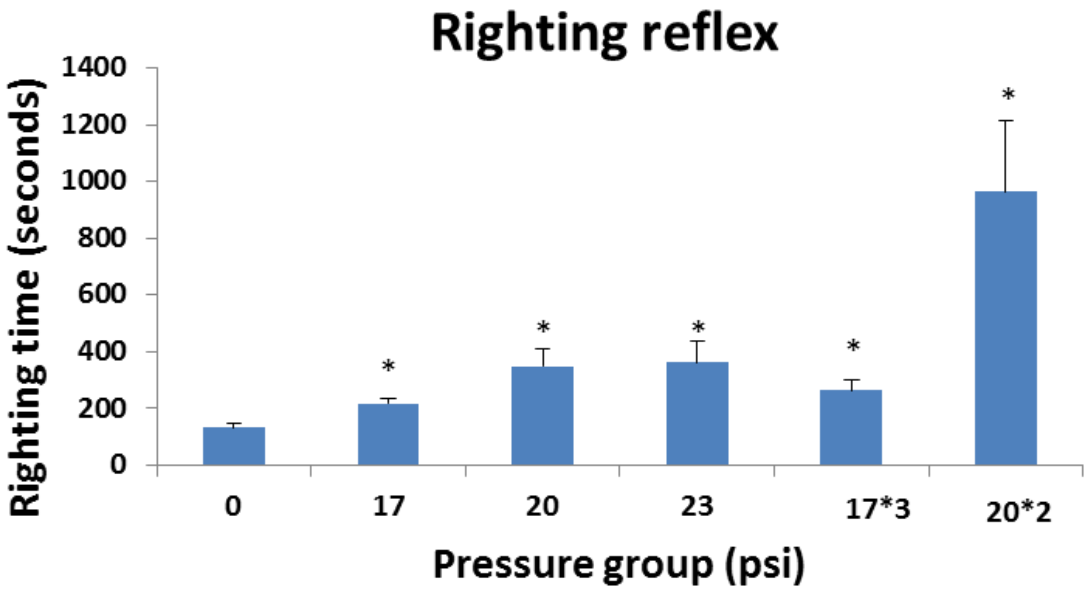

Figure 2: An increase in righting reflex time was found in all pressure groups when compared to control group (0 psi) following BOP exposure (*p<0.05).

In addition, a novel attempt was made to predict the survival rate of animals using the time of righting reflex. Interestingly, a positive significant correlation (Pearson value = 0.82; p<0.05) was found between overall mortality rate and righting reflex time (Figure 3a). However, no correlation was found between immediate mortality and righting reflex time (Figure 3b).

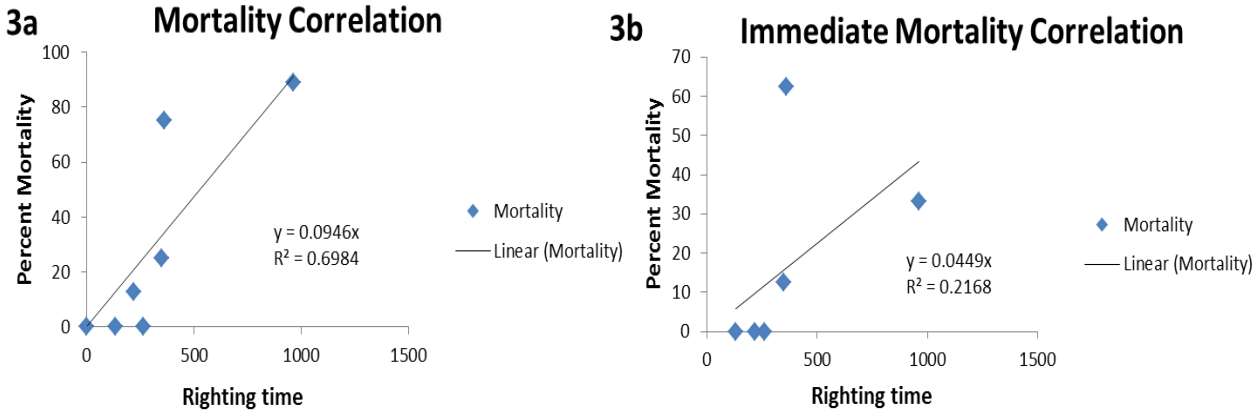

Figure 3: Panel 3a. Showing a significant correlation of overall mortality with righting flex time ($p<0.05$; $R^2 = 0.698$). However, no significant correlation was observed with immediate morality when compared to righting reflex (Panel 3b.)

3. Behavioral outcomes (Dr. Janowski)

Acute behavioral outcomes were assessed on day 2 and 3 following BOP using an open field test for overall activity of animals and thigmotaxia (anxiety-like behavior). A significant decrease in the activity was observed in the 20 and 20*2 pressure groups at day 2, while 23psi groups had significant decrease in total activity at day and 3 when compared to their respective time-point control group (Figure 4).

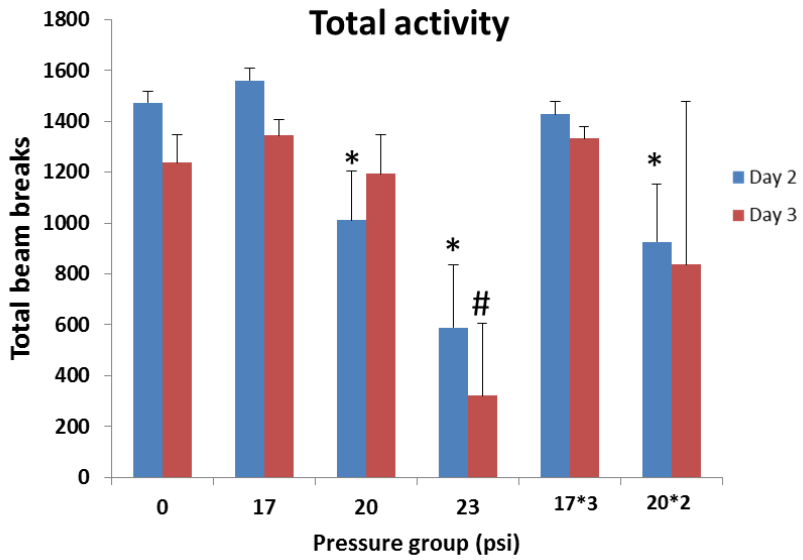

Figure 4: Graph depicting the total activity of different blast groups at days 2 and 3 (*$p < 0.05$ at day 2; #$p<0.05$ at day 3).

Increased anxiety-like behavior was observed in group in 20 psi group at day 2, 23psi group at day 2 and 3, however, decrease in anxiety-like behavior was observed in 17*3psi pressure group at day 3 (Fig. 5).

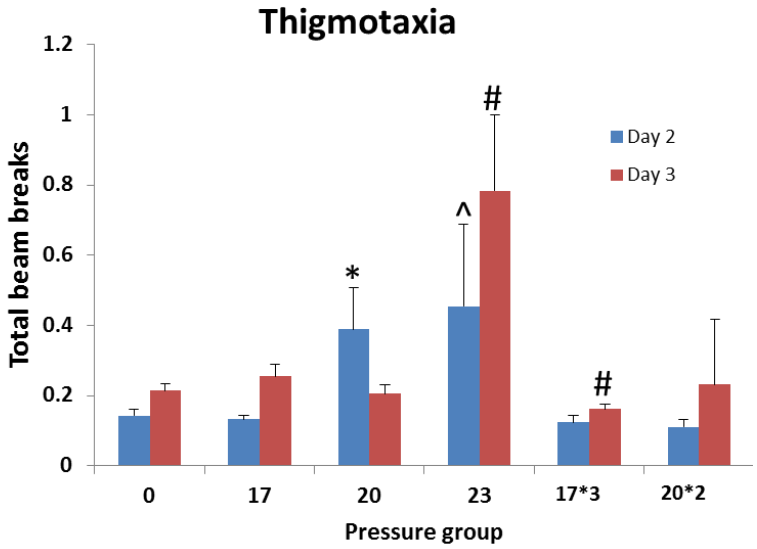

Figure 5: Anxiety-like behavior following BOP exposure (*p < 0.05 at day 2; #p < 0.05 at day 3; ^p=0.08 at day 2).

4. Ventricular Volume (Dr. Walczak)

Reduction in the ventricular volumes has been identified in all pressure groups of blast overpressure that could be the resultant of edema. Decreased volume of the ventricle (Figure 6) was observed on day 1 following BOP exposure, when the T2 weighted MRI images were segmented using image processing software AMIRA™. This could be from the potential brain swelling due to edema and increased intracranial pressure following BOP exposure, which was reported by various studies in literature.

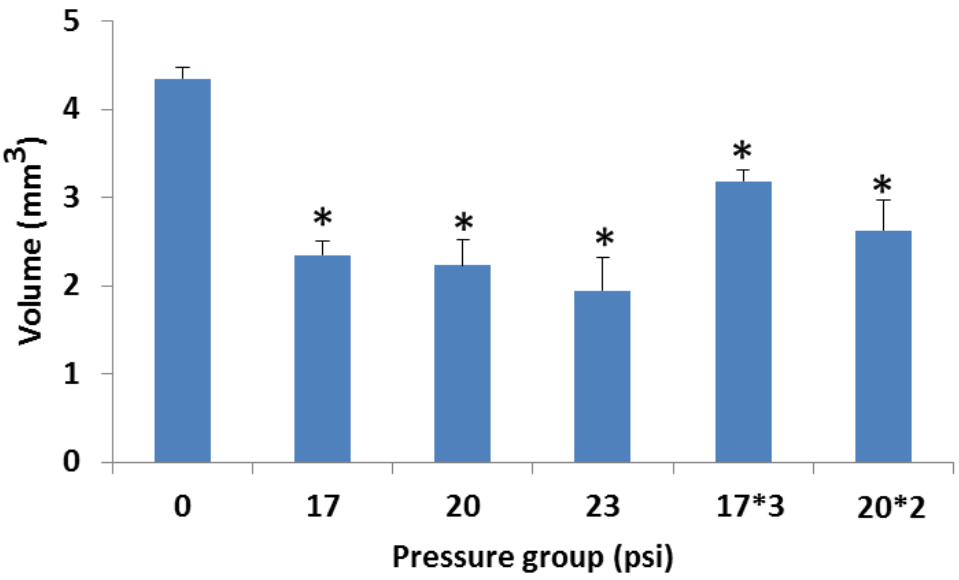

Figure 6: Decrease in ventricular volume was observed all pressure groups when compared to control group (0 psi) following BOP exposure (*p<0.001).

5. Immunohistochemistry (Dr. Janowski)

Immunohistochemistry assessment was performed to study acute apoptosis (4 days following blast overpressure). While the studies are currently underway to evaluate apoptosis in all pressure groups, thus far, we found a significant increase in levels of caspase-3, a marker of apoptosis, in the 17*3 pressure group as depicted in figure 7.

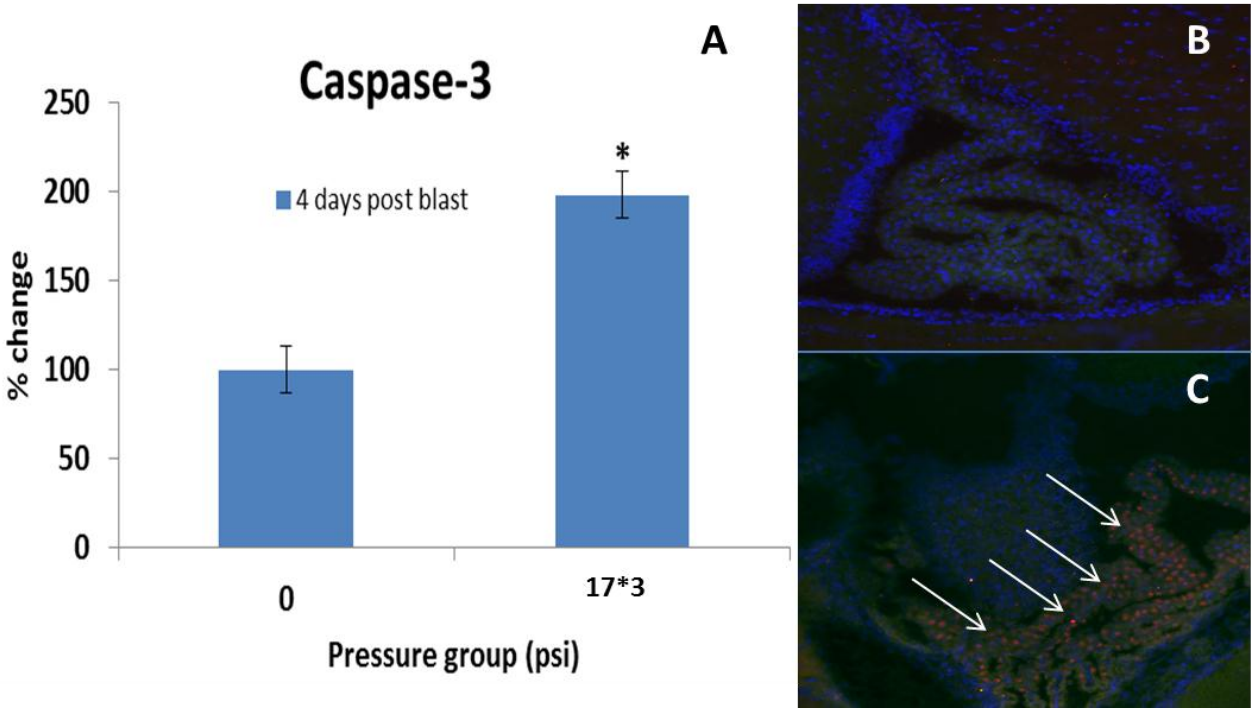

Figure 7: An increase in marker of apoptosis, caspase-3 was observed at 17*3 pressure in choroid plexus when compared to control group (0 psi) following BOP exposure (*p<0.05). Graph (A) depicts an increase in capase-3 levels, representative images of control- 0 psi (B) and 17*3 psi pressure group (C). Arrows represents caspase-3 positive cells.

6. Diffusion tensor imaging (DTI) (Dr. Walczak)

Diffusion tensor imaging depicted an increase in fractional anisotropy (FA) and axial diffusivity (AD) while no changes was observed in radial diffusivity (RD) following blast overpressure exposure (Figure 3). Although the reason for these DTI attributes is currently unknown, the pathological alternations those lead to increased FA and AD but not RD are being identified using immunohistochemistry. In addition, we have employed region of interest based assessment of white matter changes in optic tract (OT), internal capsule (IC), fimbria (Fi) and corpus callosum (CC). Furthermore, DTI slices are averaged from both control (0 psi group) and 20 psi pressure group using image processing software AMIRA™, averaged images of blast were subtracted from background of control animals to visually observe the differences as shown in figure 8.

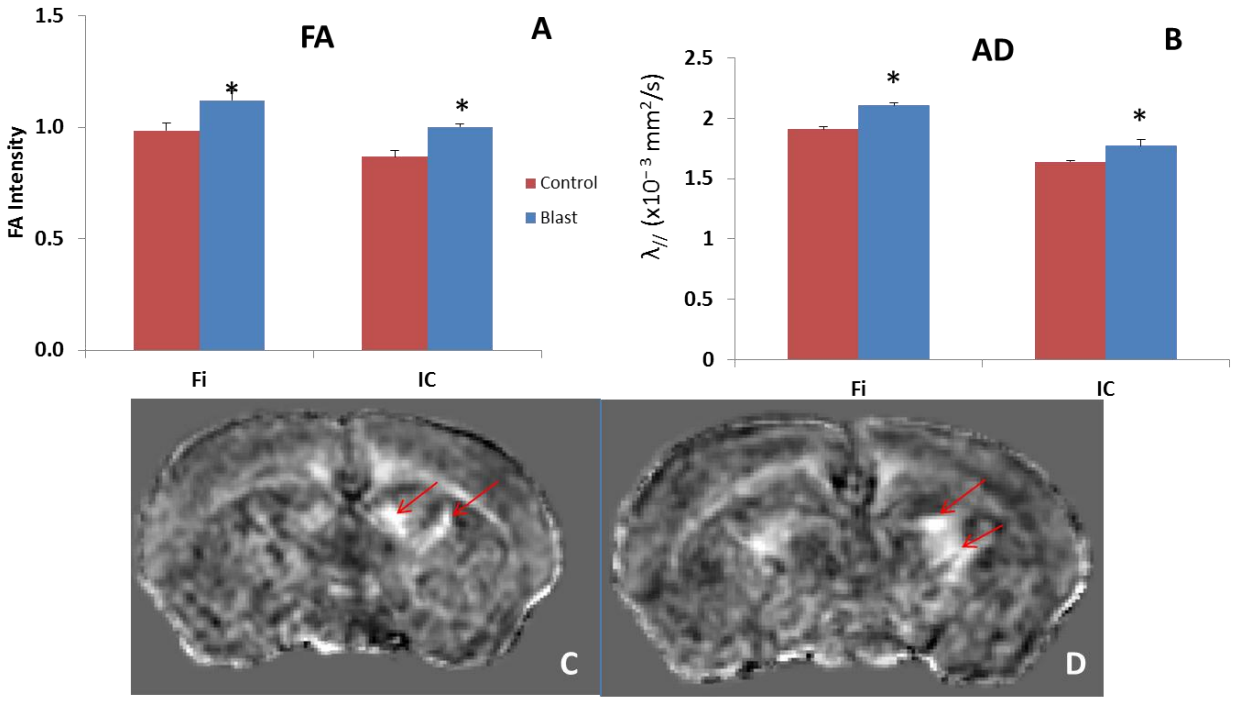

Figure 8: A significant increase in FA (A) and AD (B) was shown in Fi and IC regions of the brain at 4 days following blast overpressure exposure. C and D are representative the differential images (Avg. Blast – Avg. Control) obtained using image processing software AMIRA™.

Progress Details:

At the beginning of the project we had a kick off meeting with all co-investigators and the JHU animal resources leadership and discussed details and logistics of the animal transition between WRAIR and Johns Hopkins. This multi-institutional arrangement is also quite complex and requires careful planning and developing specific rules to strictly follow grant proposal yet without compromising animal housing quarantine regulations.

We had also identified postdoctoral fellow candidate with appropriate qualifications for spearheading the project. It has not been an easy task since the field of blast injury is still relatively small and finding candidate with right background required interviewing many candidates. Dr. Sujith Sajja from Virginia Tech was a perfect match for this project.

Information in regards to BOP exposure has been acquired. Behavior assessment, T2 and T2* weighted MRI data in addition to DTI was acquired. Base-lines of lung trauma, brain trauma responses have been identified for mice model of BOP using Balb/c strains. Histopathological and DTI studies are currently under processing stage. This information concludes the overall BOP exposure studies and leads way to future longitudinal and therapeutic studies using stem cells.

Information in regards to blast overpressure exposure optimal dosage has been acquired for chronic studies of demyelination. Chronic behavior assessment for learning and memory, temporal T2 imaging for gross anatomical abnormalities and temporal has been acquired. Acute histopathological studies are under processing stage for the data. Micromolecular changes in plasma that are specific to brain are currently being identified. This information concludes the overall blast overpressure studies for chronic behavioral and pathological outcomes thus leading way to future therapeutic studies using stem cells.

Coordination of animal blasting, behavioral and post mortem tissue assessment has been coordinated by Dr. Janowski, while MR imaging with regard to data acquisition and analysis has been coordinated by Dr. Walczak.

What opportunities for training and professional development has the project provided?

Training:
Training in MR imaging and behavioral testing for a post-doctoral fellow – Sujith Sajja. He had also a chance to learn about exosomes, lipidomics and next generation sequencing (NGS), as a part of our lab collaboration.

Professional development:
Based on his training he has prepared 2 post-doctoral grant proposals for JHMVHI Russell Scholar Program, and one of it entitled “Chemical Exchange Saturation Transfer Imaging as a Diagnostic Tool for the Identification of Mild-Moderate Traumatic Brain Injury” has been awarded to him.

How were the results disseminated to communities of interest?

During the first 6 months of the experiments it was too little time to disseminate results to community.

What do you plan to do during the next reporting period to accomplish the goals?

The analysis of outcome of long-term blast study (MRI, behavior, post mortem evaluation) in immunocompetent mice, and performing the same study on immunodeficient mice to test whether the blast injury in immunodeficient animals is the same as in immunocompetent animals. Preparation of publication from short-term blast dose finding study in mice. We have found the surprising finding of increase of fractional anisotropy (FA) on DTI images, and we plan to prepare review paper about the situation when fractional anisotropy increases and why.

Then with the ongoing DTI data acquisition, we plan to identify a time-point that is best suited for stem cell therapeutics. This leads the way to the stem cell therapeutics for demyelination.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

Dose escalation study we performed was the first systematic characterization of blast injury in mice including readouts in MRI, behavior and histopathology. We are in the process of writing a manuscript describing our observations and once published we believe this work should find great interest and be of significant impact on the field of traumatic brain injury. Until now, rats were mostly used for blast studies but the wide access to transgenic animals, makes the mice very attractive tool for studying of blast injury.

What was the impact on other disciplines?

Oligodendrocytes and myelin play important role in many neurological disorders including stroke, multiple sclerosis or even amyotrophic lateral sclerosis so knowledge about the status of myelin following TBI may bring important clues about myelin damage in these diseases.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to Report

5. Changes/Problems:

Changes in approach and reasons for change

We had issues with the reproducibility of previous identified 20psi pressure group with minimal mortality rate. We have thoroughly investigated the cause of morality and tweaked the chamber that holds the animals in blast overpressure simulator at Walter Reed Army Institute of Research. For the studies henceforth, we have identified 17*2 psi would be an optimal dosage to carry with our long-term studies. No further problems are anticipated at this point.

Actual or anticipated problems or delays and actions or plans to resolve them

The project involves use of vertebrate animals and as per DoD regulations it requires review and approval by ACURO. Not being aware about the length of the review and approval process we did not include this milestone in our timeline which required revision.
Over the initial three months we secured all required approvals including Johns Hopkins IACUC and ACURO.

Changes that had a significant impact on expenditures

In this reporting period our expenses included personnel costs, supplies, contractual services and domestic travel at the total direct costs of $122,258.94. Leaving a positive balance at $38,121.06. This slightly lower expense rate compared to budgeted is due to some initial delays related to securing all required approvals. We request that the remaining balance is transferred towards our expenses in the second year of the project.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

6. Products

Nothing to report

7. Participants & Other Collaborating Organizations

Miroslaw Janowski MD/PhD: effort 25 %

Robert Stevens MD/PhD: effort 6.98 %

Sujith Sajja PhD: effort 50 %

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Walter Reed Army Institute of Research (WRAIR)

8. Special Reporting Requirements

As instructed for this collaborative award technical portion of this progress report is duplicative with report for the grant # W81XWH-13-1-0388 with listed contribution each PI.

9. Appendices

Nothing to report